Higher silver bioavailability after nanoparticle dietary exposure in marine amphipods

Environmental Toxicology and Chemistry

Short Communication

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Environ Toxicol Chem., **Accepted Article** • DOI: 10.1002/etc.4359

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Submitted 7 November 2018; Returned for Revisions 18 December 2018; Accepted 9 January 2019

ABSTRACT

Upon release into surface waters, engineered silver nanoparticles (AgNP) tend to settle to sediments and, consequently, epibenthic fauna will be exposed to them through the diet. We established Ag uptake and accumulation profiles over time in the haemolymph of a marine amphipod fed with a formulated feed containing AgNP or AgCl. Silver bioavailability was higher in organisms exposed to AgNP, indicating that the nanoparticles pose a higher risk of toxicity compared to similar concentrations of AgCl. This article is protected by copyright. All rights reserved

Keywords: accumulation, AgNP, haemolymph; metal uptake, *Parhyale hawaiensis*, silver.

INTRODUCTION

Silver (Ag) has been used as a broad spectrum antimicrobial agent for many years and the relatively recent development of engineered Ag nanoparticles (AgNP) has expanded the use of Ag considerably (Purcell and Peters 1998; Marambio-Jones and Hoek 2010; Fabrega et al. 2011; Musee 2011). As a consequence, Ag is released into surface waters (Morgan et al. 1997; Purcell and Peters 1998) and can reach toxic concentrations to the aquatic life (Purcell and Peters 1998; Wood et al. 1999). Silver nanoparticles tend to agglomerate in the aqueous phase and settle to sediment surfaces (Forstner 1983). Epibenthic organisms including the marine amphipod *Parhyale hawaiensis* that feed on sediment surfaces can be exposed to these materials through the diet (Petersen and Henry 2012). To date, few ecotoxicology studies have been published with marine amphipods and nanomaterials (Melo and Nipper 2007; Wong et al. 2010; Fabrega et al. 2012; Petersen and Henry 2012; Hannaa et al. 2013; Wang et al. 2014; Canesi and Corsi 2016). Only one study investigated the acute effect of AgNP amended into sediments (at ≤75 mg/ kg dry wt) for 7 days to the marine amphipod *Ampelisca abdita* and no mortality was observed (Wang et al. 2014).

The mechanisms of AgNP toxicity in aquatic organisms are still unsolved. Some evidence indicates that toxicity is a consequence of the release of Ag ions upon dissolution of AgNP, whereas other studies indicate that AgNP particle-specific effects that contribute to toxicity are not explained solely by the release of Ag^+ (Ratte 1999; Park et al. 2011; George et al. 2012). Information is lacking on the relation between AgNP exposure, uptake and depuration profiles and how these relate to toxicity. To clarify this issue, the measurement of internal Ag concentrations after exposure is necessary. Metal exposure analyses in small crustaceans are made frequently by analysis of the whole animal (Fialkowski et al. 2003; Arce Funck et al. 2013; Andreï et al. 2016), however this approach cannot provide information

about the actual internal concentrations. A better approach is to examine the haemolymph to determine the amount of the Ag absorbed and distributed within the animal. Analysis of haemolymph has already been applied in studies using decapods (Grosell et al. 2002) and in freshwater invertebrates such as *Daphnia* (Zhao and Wang 2010; Scanlan et al. 2013). In 2018, Vannuci-Silva et al. (2018) developed a reliable method for measuring Ag and Cu in haemolymph of *P. hawaiensis* and showed that Ag increases in haemolymph when animals are exposed to $AgNO_3$ in solution.

The aim of this study was to investigate Ag concentration in the haemolymph of the marine amphipod *Parhyale hawaiensis* after dietary exposure to AgNP and AgCl amended food and to establish uptake and accumulation profiles of Ag in the haemolymph over time. We hypothesize that concentration profiles of Ag in the haemolymph will differ between organisms fed either with AgNP or AgCl because of the differences in Ag bioavailability between nanoparticle and salt forms.

MATERIAL AND METHODS

Parhyale hawaiensis organisms were cultivated in the Laboratory of Ecotoxicology and Genotoxicity (LAEG) of the School of Technology of the State University of Campinas, according to Artal et al. (2017). As a proof of concept to verify if Ag could be efficiently measured in the haemolymph, we performed some experiments using only Ag in salt form ($AgNO_3$) dissolved in water using different exposure times and concentrations (Pokhrel and Dubey 2012; Andreï et al. 2016). Then, feeding experiments were performed with food containing either AgCl salt or AgNP for the determination of the Ag content in haemolymph of organisms from both treatments in different times of exposure.

Materials, reagents and equipment

The reagents used were HNO_3 with 65% purity (Merck; Sigma-Aldrich); 1000 mg L^{-1} silver monoelementar standard solution (Quemis), $AgNO_3$ with ≥ 99% purity (Sigma-Aldrich) and other reagents generally used in chemical analysis. Glassware, plastic bottles and other materials used during the collection and analysis of samples were decontaminated with 10% (v/v) HNO_3 for 24 hours. All solutions, dilutions and washes were performed with ultrapure water (Millipore, 18 MΩ cm resistivity).

An Analyst 600 graphite furnace atomic absorption spectrometer (PerkinElmer, Inc., Shelton, CT, USA) was used for Ag determinations in the haemolymph, in the saline water and in the food used in the dietary exposure experiments. A 7700x Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent Technologies, Hachioji, Japan) was used for Ag determinations in the haemolymph and in the saline water from $AgNO_3$ exposure via water. Other equipment used were: magnetic shaker (Fisaton, model 753, São Paulo, Brazil); pH meter (Thermo Scientific, model Orion Star A211, Singapore); conductivity/salinity meter (Thermo Scientific, model Orion Star A212, Singapore); oxygen meter (YSI, model 55, Yellow Springs, USA), incubator with photoperiod (Marconi, model MA403 and Eletrolab, model EL 202/4, Piracicaba, Brazil) and analytical scale (Shimadzu, model AUW220D, Cebu, Philippines).

Exposure to $AgNO_3$ via water

Adult organisms were individually exposed via water to non-lethal concentrations (0; 5; 10; 25; 50 and 100 µg L^{-1} of Ag from $AgNO_3$) during 14, 24, 48, 72 and 96 hours without feeding or aeration. Twelve replicates were used with a 1:1 sex ratio for each treatment. The haemolymph was collected according to Vannuci-Silva et al. (2018), and silver concentrations were determined using an inductively coupled plasma mass spectrometer (ICP-MS). Silver

concentrations in the exposure solutions were determined at the end of experiments and a comparison between the final and the nominal concentration was done. The saline water sample was acidified with HNO_3 2% for Ag preservation. After 1:10 dilution, the sample was directly introduced into the ICP-MS using an High Matrix Introduction System (HMI) because it supports a high content of dissolved solids (until 5%) (Vannuci-Silva et al. 2018). The limit of detection (LOD) and the limit of quantification (LOQ) for the haemolymph were 0.13 and 0.44 μg L^{-1}, respectively. For the saline water, the LOD was 1.2 μg L^{-1} and the LOQ was 4.1 μg L^{-1}, as described by Vannuci-Silva et al. (2018).

Exposure to AgNP and AgCl via food

Feeding exposure experiments were conducted with two types of food, one containing AgNP and another with Ag salt (AgCl). A control group was fed with the same basal diet used in the contaminated food (ca. 40% protein and 6% lipid). The contaminants were incorporated into the basal diet by adding the unmodified powdered form within the feed pellets as described by Merrifield (2013). The AgNP used in this study were obtained from Sigma-Aldrich (nano <100 nm) with a mean particle diameter of 58.6 ±18.6 nm (mean ± S.D., n = 64) and were from the same batch as reported in the works of Bradford et al. (2009) and Merrifield et al. (2013). Silver as AgCl (Sigma-Aldrich, with 99% purity) was also added to the basal diet to provide a feeding treatment with an Ag inorganic form. The metal concentration in both food was evaluated by graphite furnace atomic absorption spectrometry (GF AAS) analysis. The determinations indicated that Ag concentrations were 155 mg kg^{-1} and 195 mg kg^{-1} in the food preparations for AgNP and AgCl, respectively.

Adult animals were individually allocated in plastic containers with 100 mL of reconstituted saline water (30±2 salinity). They were fed with control food or with food containing either AgNP or AgCl pellets. After 1 hour of feeding, organisms were rinsed with

ultrapure water and placed in new plastic containers and clean artificial saline water to ensure that the exposure was only via food. The times of exposure were 7, 14 and 28 days. The tests were carried out at the same conditions of salinity, temperature and photoperiod as used in the husbandry (30, 24±2°C and 12h light-12h dark, respectively) but without aeration. Twelve replicates were used for each treatment, with a 1:1 sex ratio and the experiment was performed twice. In the first experiment, the organisms were fed daily. As it was observed that not all organisms ate every day, a second experiment was carried out with the same conditions but with food provided on alternate days.

Silver determination in haemolymph was carried out by GF AAS as described by Vannuci-Silva et al. (2018). The measurements were performed using the wavelength of 328.1 nm and samples and chemical modifier (Pd 5µg/$Mg(NO_3)_2$ 3µg) volumes injected into the graphite tube were 20 µL and 5 µL, respectively. Silver concentrations in saline water of the exposure assays were determined in 17 samples at the beginning and at the end of one hour of feeding, to ensure that no Ag was present in the water. The saline water sample was acidified with HNO_3 2% for Ag preservation. Samples were directly introduced into the GF AAS after 1:10 dilution. The LOD and the LOQ for Ag in the haemolymph were 0.11 and 0.37 ng mg^{-1}, respectively. The LOD and LOQ for Ag in saline water were 0.16 and 0.54 µg L^{-1}, respectively (Vannuci-Silva et al. 2018).

Haemolymph collection

Haemolymph was obtained as described by Vannuci-Silva et al. (2018). The fluid was collected using a thin glass needle that was manually made from a capillary glass. Using tweezers, the animals were immobilized and placed with the dorsal segments clearly visible on a decontaminated glass plate. The needle was inserted into the first or second dorsal segment and the haemolymph was extracted with the capillary needle. The amount of

haemolymph collected was placed into a 2 mL Eppendorf tube containing 0.5 or 1 mL of HNO_3 0.05%. Each capillary glass was weighed before and after the haemolymph collection. We collected 0.87±0.42 mg of haemolymph per animal (n=574). Three pooled samples of 4 organisms (2 females and 2 males) were tested per treatment.

Uptake rates

Silver concentrations were measured in the haemolymph of animals exposed either via water or diet. The uptake kinetics were modelled as described by Reinardy et al.(2011), with a single-component first order kinetic model (1):

(1) $C_t = C_{ss}(1 - e^{-ket})$

where C_t and C_{ss} represent activity concentration at time t (d) and at steady state, respectively, and k_e represents biological uptake rate constant (d^{-1}). If there was no indication of reaching a steady state during the time of exposure (non-significant fit to the model above), a simple linear regression model was applied (2):

(2) $C_t = k_u t$

where k_u is the slope of regression.

Expression of results and statistics

The results were reported as the mean ± the standard deviation (SD) and expressed in mg kg^{-1} for the Ag concentration in the food, µg L^{-1} for Ag concentration in the water and in ng mg^{-1} for Ag concentration in the haemolymph.

Silver concentration data sets were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene test). When data were acceptable, analyses of variance (ANOVA) were performed to assess the relation between Ag haemolymph concentration and the independent factors: Ag concentration in the water/food and exposure time. A Tukey test

was posteriorly applied. The IBM SPSS statistics program (version 24) was used for all statistical analyses and p values of <0.05 were considered significant.

Parameters and statistics of uptake models were estimated by iterative adjustments by non-linear exponential rise to maximum and linear functions in SigmaPlot 12.5 (Systat Software, San Jose, CA). Where the significance of the model was satisfied ($p<0.05$), the model was applied to the data.

RESULTS AND DISCUSSION

Exposure to $AgNO_3$ via water

The Ag determinations in the saline water, at the end of the experiments, were 72-87% of the Ag concentrations added at the beginning of the experiment. Silver concentration in the haemolymph increased with exposure time and silver concentration in the water (Figure 1), differences were significant between exposure times ($p<0,001$), concentrations ($p<0,001$) and their interaction ($p<0,001$). However, no significant difference between 50 and 100 µg L^{-1} treatments were found ($p=0.088$).

We calculated uptake rates for each Ag water concentration tested. The general equation that fitted all data was $f=y^0+a*(1-\exp(-b*x))$. The *a* and *b* coefficients for each concentration, the slope correlation (R^2) and the p value for the regressions are described in Table 1.

The angular coefficient (*a*) in the equations increased proportionally to the increase of silver concentration in the water, up to 50 µg L^{-1}. Above 50 µg L^{-1}, the uptake rate reached a plateau, suggesting some mechanism of regulation when Ag concentrations in water reaches

this amount. The proposed equation can be used to estimate the Ag concentration in the haemolymph of organisms exposed to Ag via water.

Exposure to AgNP and AgCl via food

Data from animals fed daily and fed on alternate days were combined and analysed together because they were not statistically different (p= 0.177). Ag was not released from food to the water media during the experiments (concentrations in the water were lower than the LOQ), therefore, the only source of Ag was the diet.

There was no difference in the Ag concentration in the haemolymph among the organisms in the control group at different exposure times (p=0.963). Ag concentrations in the haemolymph for AgCl food exposed organisms showed no difference between 7 and 14 days of exposure (p= 0.784) and they were 1.3±0.2 and 1.6±0.3 ng mg^{-1}, respectively. However, for 28 days of exposure, an Ag increase was observed, reaching 3.7±1.0 ng per mg of haemolymph (p>0.001 when compared to 7 and 14 days). Silver concentrations in the haemolymph for AgNP food exposure during 7 and 14 days were 3.3±2.0 and 5.1±0.2 ng mg^{-1}, respectively, and there was no significant difference between these values (p=0.271), probably due to the high standard deviation observed in the measurements for 7 days-exposure organisms. Nevertheless, at 28 days, Ag concentration in the haemolymph reached 8.4±0.7 ng mg^{-1}, and it was significantly higher than the 7 (p=0.001) and the 14 (p=0.027) exposure days.

Silver concentrations in the haemolymph of organisms exposed to AgNP were higher than the ones exposed to AgCl along time (Figure 2), although Ag concentration in food containing AgCl was slightly higher than in the AgNP containing food, highlighting the higher bioavailability of Ag from AgNP. No difference was observed between the two food treatments on day 7 of exposure (p=0.07), possibly because of the higher standard deviation

observed for AgNP. The haemolymph of organisms fed with AgNP food showed significant higher Ag concentration than of those fed with AgCl on 14 ($p=0.001$) and 28 ($p=0.001$) days of exposure.

Silver uptake from AgNP food is 2.8 times higher than from AgCl (Figure 2). More studies are required to verify mechanisms involved and risks associated with AgNP and AgCl exposures.

CONCLUSIONS

To the best of our knowledge, this is the first study to demonstrate and to quantify silver in the haemolymph of amphipods exposed to silver nanoparticles and two silver salts. This work establishes uptake and accumulation profiles of Ag exposure in a circumtropical distribution marine amphipod and highlights the importance of the measurement of internal concentration to study metallic nanoparticles toxicity in aquatic organisms. Silver concentration in the haemolymph of organisms fed with AgNP food was higher when compared to exposure with similar concentrations of AgCl, indicating higher Ag bioavailability from AgNP. Therefore, risks of toxicity from AgNP are higher than similar concentration of AgCl.

ACKNOWLEDGEMENTS

The authors thank FAPESP and CAPES (grant 2013/26301-7, São Paulo Research Foundation - FAPESP), National Council of Research - CNPq (grant 400362/2014-7 and 552120/2011-1), National Institute of Science and Advanced Analytical Technologies (INCTAA) and National Institute of Science, Technology and Information in Functional Complexes Materials (INOMAT) for financial support. We also thank Dr Rhaul de Oliveira and MSc Amanda dos Santos for the suggestions and contributions.

Declarations of conflict of interest:

None.

Data availability statement:

The authors will provide the data if requested via e-mail.

REFERENCES

Andreï J, Pain-Devin S, Felten V, Devin S, Giambérini L, Mehennaoui K, Cambier S, Gutleb AC, Guérold F. 2016. Silver nanoparticles impact the functional role of Gammarus roeseli (Crustacea Amphipoda). Environ Pollut. 208:608–618. doi:10.1016/j.envpol.2015.10.036.

Arce Funck J, Danger M, Gismondi E, Cossu-Leguille C, Guerold F, Felten V. 2013. Behavioural and physiological responses of Gammarus fossarum (Crustacea Amphipoda) exposed to silver. Aquat Toxicol. 142–143:73–84. doi:10.1016/j.aquatox.2013.07.012.

Artal MC, dos Santos A, Henry TB, Umbuzeiro G de A. 2017. Development of an acute toxicity test with the tropical marine amphipod Parhyale hawaiensis. Ecotoxicology. doi:10.1007/s10646-017-1875-3.

Bradford A, Handy RD, Readman JW, Atfield A, Muhling M. 2009. Impact of silver nanoparticle contamination on the genetic diversity of natural bacterial assemblage in estuarine sediments. Environ Sci Technol. 43(12):4530–4536.

Canesi L, Corsi I. 2016. Effects of nanomaterials on marine invertebrates. Sci Total Environ. 565:933–940. doi:10.1016/j.scitotenv.2016.01.085.

Fabrega J, Luoma SN, Tyler CR, Galloway TS, Lead JR. 2011. Silver nanoparticles: Behaviour and effects in the aquatic environment. Environ Int. 37(2):517–531. doi:10.1016/j.envint.2010.10.012.

Fabrega J, Tantra R, Amer A, Stolpe B, Tomkins J, Fry T, Lead JR, Tyler CR, Galloway TS. 2012. Sequestration of zinc from zinc oxide nanoparticles and life cycle effects in the sediment dweller amphipod Corophium volutator. Environ Sci Technol. 46(2):1128–1135. doi:10.1021/es202570g.

Fialkowski W, Rainbow PS, Smith BD, Zmudzinski L. 2003. Seasonal variation in trace metal concentrations in three talitrid amphipods from the Gulf of Gdansk, Poland. J Exp Mar Bio Ecol. 288:81–93. doi:10.1016/S0022-0981(02)00594-4.

Forstner U. 1983. Metal transfer between solid and aqueous phases. In: U. Forstner & G.T.W. Wittmann. In: Metal Pollution in the Aquatic Environmental. Berlin: Berlin Heidelberg:Springer-Verlag. p. 197.

George S, Lin S, Ji Z, Thomas CR, Li L, Mecklenburg M, Meng H, Wang X, Zhang H, Xia T, et al. 2012. Surface defects on plate-shaped silver nanoparticles contribute to its hazard potential in a fish gill cell line and zebrafish embryos. ACS Nano. 6(5):3745–3759. doi:10.1021/nn204671v.

Grosell M, Brauner CJ, Kelly SP, McGeer JC, Bianchini A, Wood CM. 2002. Physiological responses to acute silver exposure in the freshwater crayfish (Cambarus diogenes diogenes)--a model invertebrate? Environ Toxicol Chem. 21(2):369–374.

Hannaa SK, Miller RJ, Zhou D, Keller AA, Lenihan HS. 2013. Accumulation and toxicity of metal oxide nanoparticles in a soft-sediment estuarine amphipod. Aquat Toxicol. 142–143:441–446.

Marambio-Jones C, Hoek EM V. 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J Nanoparticle Res. 12(5):1531–1551. doi:10.1007/s11051-010-9900-y.

Melo S, Nipper M. 2007. Sediment toxicity tests using the burrowing amphipod Tiburonella viscana (Amphipoda: Platyischnopidae). Ecotoxicol Environ Saf. 66:412–20.

Merrifield DL, Shaw BJ, Harper GM, Saoud IP, Davies SJ, Handy RD, Henry TB. 2013. Ingestion of metal-nanoparticle contaminated food disrupts endogenous microbiota in zebrafish (Danio rerio). Environ Pollut. 174:157–163. doi:10.1016/j.envpol.2012.11.017.

Morgan IJ, Henry RP, Wood CM. 1997. The mechanism of acute silver nitrate toxicity in freshwater rainbow trout (Oncorhynchus mykiss) is inhibition of gill Na+ and Cl- transport. Aquat Toxicol. 38(1–3):145–163. doi:10.1016/S0166-445X(96)00835-1.

Musee N. 2011. Nanowastes and the environment: Potential new waste management

paradigm. Environ Int. 37(1):112–128. doi:10.1016/j.envint.2010.08.005.

Park MVDZ, Neigh AM, Vermeulen JP, de la Fonteyne LJJ, Verharen HW, Briedé JJ, van Loveren H, de Jong WH. 2011. The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials. 32(36):9810–9817. doi:10.1016/j.biomaterials.2011.08.085.

Petersen EJ, Henry TB. 2012. Methodological considerations for testing the ecotoxicity of carbon nanotubes and fullerenes: Review. Environ Toxicol Chem. 31(1):60–72. doi:10.1002/etc.710.

Pokhrel LR, Dubey B. 2012. Potential impact of low-concentration silver nanoparticles on predator-prey interactions between predatory dragonfly nymphs and Daphnia magna as a prey. Environ Sci Technol. 46(14):7755–7762. doi:10.1021/es204055c.

Purcell TW, Peters JJ. 1998. Sources of silver in the environment. Environ Toxicol Chem. 17(4):539–546. doi:10.1002/etc.5620170404.

Ratte HT. 1999. Bioaccumulation and toxicity of silver compounds: A review. Environ Toxicol Chem. 18(1):89–108. doi:Doi 10.1897/1551-5028(1999)018<0089:Batosc>2.3.Co;2.

Reinardy HC, Teyssie J, Jeffree RA, Copplestone D, Henry TB, Jha AN. 2011. Uptake, depuration and radiation dose estimation in zebra fi sh exposed to radionuclides via aqueous or dietary routes. Sci Total Environ. 409(19):3771–3779. doi:10.1016/j.scitotenv.2011.06.057.

Scanlan LD, Reed RB, Loguinov A V., Antczak P, Tagmount A, Aloni S, Nowinski DT, Luong P, Tran C, Karunaratne N, et al. 2013. Silver nanowire exposure results in internalization and toxicity to Daphnia magna. ACS Nano. 7(12):10681–10694. doi:10.1021/nn4034103.

Vannuci-Silva M, do Nascimento da Silva E, Coletty M, dos Santos A, Silva F, Umbuzeiro GDA, Cadore S. 2018. GFAAS and ICP-MS determination of Ag and Cu in the haemolymph

of a millimetric marine crustacean (Parhyale hawaiensis) as a Tool in Ecotoxicology. At Spectrosc. 39(2):67–74.

Wang H, Ho KT, Scheckel KG, Wu F, Cantwell MG, Katz DR, Horowitz DB, Boothman WS, Burgess RM. 2014. Toxicity, bioaccumulation, and biotransformation of silver nanoparticles in marine organisms. Environ Sci Technol. 48(23):13711–13717. doi:10.1021/es502976y.

Wong SWY, Leung PTY, Djurišić AB, Leung KMY. 2010. Toxicities of nano zinc oxide to five marine organisms: Influences of aggregate size and ion solubility. Anal Bioanal Chem. 396(2):609–618. doi:10.1007/s00216-009-3249-z.

Wood CM, Playle RC, Hogstrand C. 1999. Physiology and modeling of mechanisms of silver uptake and toxicity in fish. Environ Toxicol Chem. 18(1):71–83. doi:10.1897/1551-5028(1999)018<0071:pamomo>2.3.co;2.

Zhao CM, Wang WX. 2010. Biokinetic uptake and efflux of silver nanoparticles in Daphnia magna. Environ Sci Technol. 44(19):7699–7704. doi:10.1021/es101484s.

Figure captions

Figure 1. Silver concentration (ng mg^{-1}) in the haemolymph of *Parhyale hawaiensis* organisms exposed to Ag via water at different exposure times. Silver concentrations in the control group were not statistically different regarding time and are not shown in this figure. Determination was carried out by ICP-MS.

Figure 2. Silver concentration (ng mg^{-1}) in the haemolymph of *Parhyale hawaiensis* organisms fed with AgNP (open circles) and AgCl (filled circles) for 0, 7, 14 and 28 days. Silver concentrations in the control group were not statistically different regarding time and are not shown in this figure. Determination was carried out by GF AAS.

Table 1. Uptake rates coefficients for exposure to different concentrations of Ag salt ($AgNO_3$) via water.

Ag in water, µg L^{-1}	***a***	***b***	**R^2**	**p**
5	$3.18x10^{7}$	5.07^{-10}	0.5864	0,0013
10	2.88	0.029	0.8806	<0,0001
25	8.09	0.039	0.9252	<0,0001
50	11.01	0.050	0.9666	<0,0001
100	9.97	0.063	0.9165	<0,0001

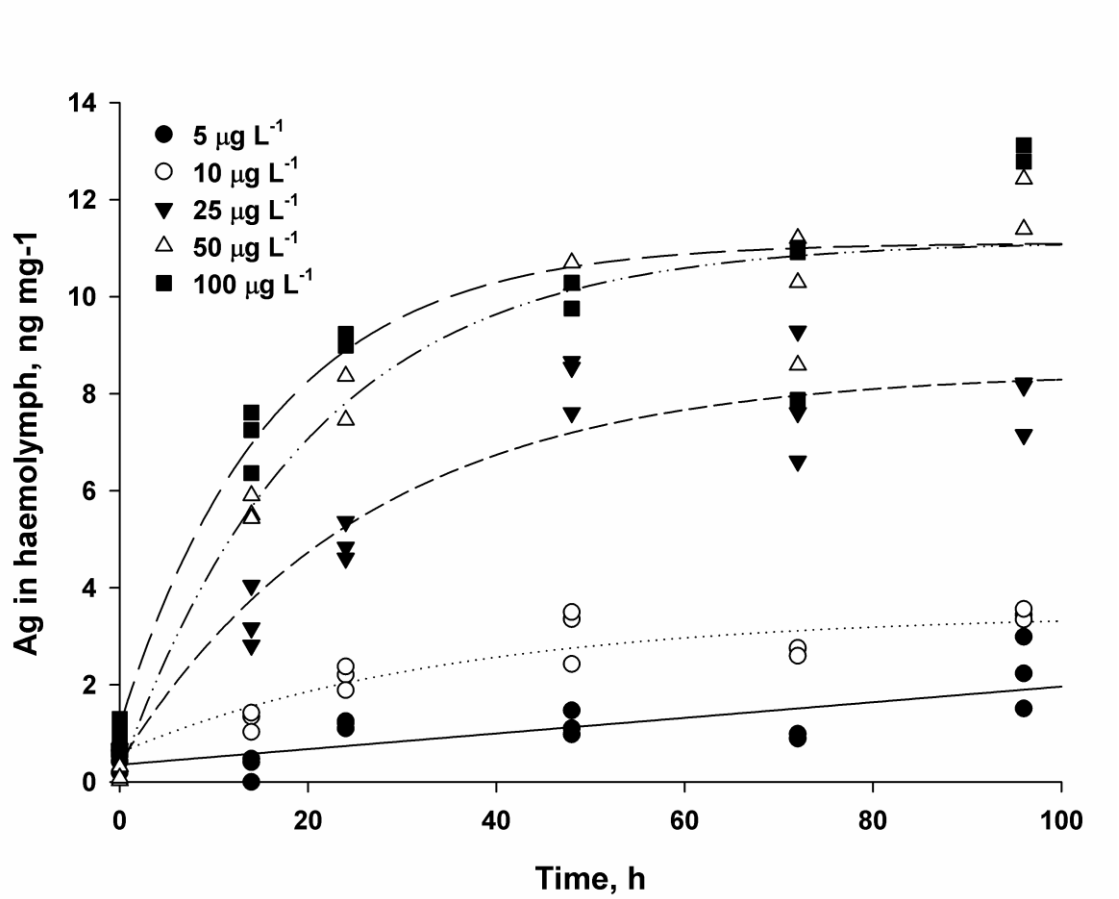

Figure 1

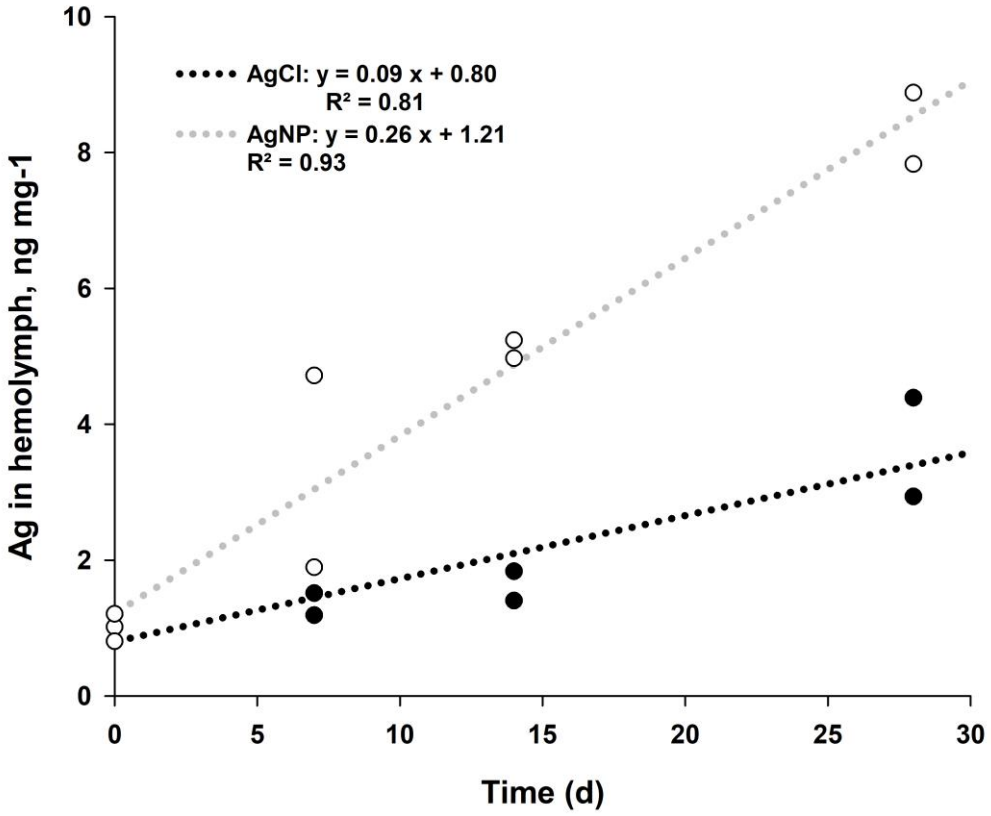

Figure 2